REMARKS

Amendments

Claims 1, 3, 5, 7, 8 and 9 have been amended to recite that the claimed polypeptides "specifically bind to an anti-*Ehrlichia* antibody." Support for the amendments can be found, *inter alia*, at page 9, lines 8-15. Claim 2 inadvertently depended from itself, so it has been amended to properly depend from claim 1. Claims 3, 5, 8 and 9 have been amended to recite polypeptides "consisting essentially of" the polypeptides of SEQ ID NO:2 or variants thereof.

All amendments are made without prejudice, and the Applicants reserve the right to pursue the subject matter in a continuation application. No new matter is added by these amendments, and Applicants respectfully request their entry.

The Applicants wish to thank the Examiner for withdrawing the objections to the specification and claims as outlined in the Office Action of July 3, 2002. The Applicants also wish to thank the Examiner for withdrawing the rejection of claim 1-6 under 35 U.S.C. § 102(b) as related to the reference by Zhi et al.

Rejection of Claims 1-9 Under 35 U.S.C. §112, first paragraph

Claims 1-9 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking written description. Applicants respectfully traverse the rejection.

As amended, each of the independent claims now recites a polypeptide "consisting essentially of" the about 20 amino acid polypeptide of SEQ ID NO:2, phenotypically silent amino acid substitution variants of SEQ ID NO:2, or a conservative amino acid substitution variants of SEQ ID NO:2, that specifically bind to an anti-*Ehrlichia* antibody. As Applicants have previously explained, the specification provides extensive teaching regarding what are "variants" of SEQ ID NO:2 (see, e.g., page 5, lines 7-14), what is identity (see, e.g., page 6, line 3 through page 7, line 5), what are phenotypically silent and conservative amino acid substitution variants of the invention (see, e.g., page 7, line 12 through page 9, line 7, *citing* Bowie, et al., *Science*, 247: 1306 (1990)[1], which

[1] A copy of this reference was attached to the Applicants' Response to the Office Action of July 3, 2002.

teaches methods of construction of variants and the tolerance of protein sequences of substitutions), and how to make and screen the variants (see, e.g., page 18, line 19 through page 19, line 13; page 7, line 6 through page 9, line 7). In addition, variations can be made in a polypeptide shown in SEQ ID NO:2 without affecting antigenicity. See specification page 8, lines 9-20 (teaching that proteins are surprisingly tolerant of amino acid substitutions and providing guidance to the types of amino acid substitutions that are well tolerated).

The Final Office Action asserts that the Applicants have provided no structural description or detailed chemical structure accompanying the variant language recited in the claims. The Applicants respectfully disagree. In the example of variants of SEQ ID NO:2, the specification recites that the variants of the invention comprise at least 85% identity to, in this case, SEQ ID NO:2. Where SEQ ID NO:2 exists as a monomer (i.e., not repeated), three substitutions are allowed according to the specification. By definition, a very significant amount of structural description and a significantly detailed chemical structure is provided by way of the 85% or more of the sequence described by SEQ ID NO:2 that is unperturbed in the variants. Combined with the knowledge of one of skill in the art pertaining to the kinds of substitutions that are considered conservative (see, for example, Johnson MS, *et al,* Journal of Molecular Biology. 233: 716-738 (1993); Karlin, S., *et al.,* Proc. Natl. Acad. Sci. USA. 87: 2264-2268 (1990); and Altschul, SF, Journal of Molecular Biology, 219: 555-665 (1991); attached hereto), and the teaching in the specification (e.g., page 7, line 6 through page 9, line 7), the specification clearly demonstrates that Applicants possessed conservative amino acid substitution variants of SEQ ID NO:2. Further, combined with the teaching in the specification, and in Bowie *et al.* that proteins are surprisingly tolerant to substitutions in general, the specification clearly demonstrates that Applicants had possession of phenotypically silent variants (*e.g.* page 8, lines 3-20 and Bowie *et al.*).

The Final Office Action also asserts that it is not routine in the art to screen for multiple substitutions, that the positions within the polypeptide's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining similar activity are limited in any polypeptide, and that the result of such modifications is unpredictable based on the instant

disclosure. However, it appears that Examiner has failed to consider the large body of art pertaining to amino acid substitutions. See, for example, the attached references by Bowie, *et al*, Karlin, *et al*, and Altschul (*supra)*. These references are simply examples of the information available to one of skill in the art to show how to substitute amino acids with a reasonable expectation of success.

Moreover, while one of skill in the art would know that catalytic centers are more sensitive to substitutions than amino acids that are uninvolved in catalysis, one of skill in the art would also know that conservative (and non-conservative) substitutions can even be made in and directly around enzyme catalytic centers while maintaining their activity utilizing information known in the art. *See, e.g.*, Knowles, JR, Science, 236 (4806): 1252-8 (1987). Sensitivity to substitutions is in large part based on upset of the packing of tertiary and quaternary structure present in most full size proteins. See, e.g., Bowie, page 1307. Branden & Tooze, Introduction to Protein Structure, Garland Publishing 1991 (copy of pages 12, 15 are attached) teach that the average length of helix is 10 amino acids, and a single strand of beta sheet is 7 amino acids. Thus, one of skill in the art would expect that tertiary structure is minimal in short sequences This is especially relevant for the about 20 amino acid sequences of the invention, because tertiary structure is minimal to nonexistent and quaternary structure is nonexistent. Consequently, concerns about disruption of function by amino acid substitution, for example, through disruption of amino acid packing at the core of a folded protein are minimized for the about 20 amino acid polypeptides of the invention

Still further, those of skill in the art would know that, as Bowie observed in 1990, "An amino acid sequence encodes a message that determines the shape and function of a protein. This message is highly degenerate in that many different sequences can code for proteins with essentially the same structure and activity." Bowie, *et al.* p. 1306. Thus, contrary to the assertions of the Final Office Action, the practitioner would expect that substitutions could be made to the polypeptide sequence with a reasonable expectation of success, i.e., that the resulting sequence binds to anti-*Ehrlichia* antibodies.

Furthermore, making and testing the polypeptides and variants of the invention are trivial as outlined in the specification at, *inter alia*, page 10, line 6 through page 11, line 6; page 11, line 21

through page 16, line 8; Example 1, page 17, line 11 through page 19, line 13. Thus, it is trivial and routine to screen for the substitutions possible while maintaining 85% or greater identity according to the specification and there is a reasonable expectation of success in so doing. Consequently, one of skill in the art would know that the specification provides sufficient disclosure allowing her to clearly recognize that the Applicants invented what is claimed. See *in re Gostelli*, 872 F.2d 1008, 1012 (Fed. Cir. 1989).

Given the teaching of the specification and the prior art, it is clear that Applicants were in possession of polypeptides consisting essentially of the about 20 amino acid polypeptide of SEQ ID NO:2 or phenotypically silent or conservative amino acid substitution variants of SEQ ID NO:2 that specifically bind to an anti-*Ehrlichia* antibody. Thus, the specification fulfills the written description requirement of 35 U.S.C. § 112, first paragraph. Accordingly, Applicants respectfully request withdrawal of the rejection.

Rejection of Claims 1-9 Under 35 U.S.C. §112, first paragraph

Claims 1-9 stand rejected under 35 U.S.C. §112, first paragraph as allegedly lacking enablement. Applicants respectfully traverse the rejection.

The Final Office Action asserts that the specification does not provide enablement for a composition or an article of manufacture that comprise variants of SEQ ID NO:2. Under 35 U.S.C. § 112, all that is required is that the specification describe the invention in such terms as to enable a person skilled in the art to make and use the invention. Specifically, The test of enablement is whether one reasonably skilled in the art (1) could make and use the invention (2) from the disclosures in the patent coupled with information known in the art (3) without undue experimentation. *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988); *United States v. Telectronics, Inc.*, 857 F.2d 778 (Fed. Cir. 1988); M.P.E.P. § 2164.01. Thus, in the instant case, the specification must teach one skilled in the art how to make and use polypeptides *consisting essentially of* the about 20 amino acid polypeptide of SEQ ID NO:2 or phenotypically silent or conservative amino acid substitution variants of SEQ ID NO:2 that specifically bind to an anti-*Ehrlichia* antibody.

The specification provides ample guidance for one to make and use the invention. For example, the specification provides extensive teaching regarding making and screening or using the polypeptides of the invention (see e.g., page 7, line 14 through page 10, line 13 (making) and, e.g., page 11, line 21 through page 16, line 8; Example 1; and page 9, line 8 through page 10, line 5; page 11, line 21 through page 21, line 16 (using/screening); and, e.g., Bowie, *et al*, Johnson *et al*, Karlin, *et al*, and Altschul (*supra*)). As a whole, one of skill in the art would know how to make and use polypeptides consisting essentially of the about 20 amino acid polypeptide of SEQ ID NO:2 or phenotypically silent or conservative amino acid substitution variants of SEQ ID NO:2 that specifically bind to an anti-*Ehrlichia* antibody.

The Final Office Action asserts that the Applicants have not provided any structural description for the variants. The Applicants respectfully disagree. In the example of variants of SEQ ID NO:2, the specification recites that the variants of the invention comprise at least 85% identity to, in this case, SEQ ID NO:2. Where SEQ ID NO:2 exists as a monomer (i.e., not repeated), three conservative substitutions are allowed according to the specification. By definition, a very significant amount of structural description and a significantly detailed chemical structure is provided by way of the 85% or more of the sequence described by SEQ ID NO:2 that is unperturbed in the variants. Combined with the knowledge of one of skill in the art pertaining to the kinds of substitutions that are considered conservative (*see, e.g.* Johnson *et al.*, Karlin *et al,* and Altschul, *supra*), and the teaching in the specification (e.g., page 7, line 6 through page 9, line 7), the practitioner is provided with full enablement to make and use the conservative substitution variants as claimed. Further, combined with the teaching in the specification, and in Bowie *et al.*, that proteins are quite tolerant to substitutions and the knowledge that producing polypeptides of lengths contemplated in the invention is routine, the practitioner is provided with full enablement to make and use the phenotypically silent variants as claimed.

The Final Office Action also asserts that it is not routine in the art to screen for multiple substitutions, that the positions within the polypeptide's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining similar activity are limited in any

polypeptide, and that the result of such modifications is unpredictable based on the instant disclosure. This assertion, however, is contrary to the referenced cited herein that clearly provide the skilled artisan with information sufficient to allow the artisan to succeed in producing variants of SEQ ID NO: 2 that bind to an anti-*Ehrlichia* antibody. As mentioned above, while one of skill in the art would know that catalytic centers are more sensitive to substitutions than amino acids that are uninvolved in catalysis, one of skill in the art would also know that conservative (and non-conservative) substitutions can even be made in and directly around enzyme catalytic centers while maintaining their activity utilizing information known in the art. *See, e.g.*, Knowles, JR, Science, 236 (4806): 1252-8 (1987). Sensitivity to substitutions is in large part based on upset of the packing of tertiary and quaternary structure present in most full size proteins. See, e.g., Bowie, page 1307. Branden & Tooze, Introduction to Protein Structure, Garland Publishing 1991 (copy of pages 12, 15 are attached) teach that the average length of helix is 10 amino acids, and a single strand of beta sheet is 7 amino acids. Thus, one of skill in the art would expect that tertiary structure is minimal in short sequences This is especially relevant for the about 20 amino acid sequences of the invention, because tertiary structure is minimal to nonexistent and quaternary structure is nonexistent. Consequently, concerns about disruption of function by amino acid substitution, for example, through disruption of amino acid packing at the core of a folded protein are minimized for the about 20 amino acid polypeptides of the invention.

Still further, those of skill in the art would know that, as Bowie observed in 1990, "An amino acid sequence encodes a message that determines the shape and function of a protein. This message is highly degenerate in that many different sequences can code for proteins with essentially the same structure and activity." Bowie, *et al.* p. 1306. Thus, contrary to the assertions of the Final Office Action, the practitioner would expect that substitutions could be made to the polypeptide sequence with a reasonable expectation of success., i.e., that the resulting sequence binds to anti-*Ehrlichia* antibodies.

Furthermore, making and testing the polypeptides and variants of the invention are trivial as outlined in the specification at, *inter alia*, page 10, line 6 through page 11, line 6; page 11, line 21

through page 16, line 8; Example 1, page 17, line 11 through page 19, line 13. Thus, it is trivial and routine to screen for the substitutions possible while maintaining 85% or greater identity according to the specification and there is a reasonable expectation of success in so doing.

Thus, the specification is enabled for the full scope of the claims and therefore meets the enablement requirement of 35 U.S.C. § 112, first paragraph. Consequently, Applicants respectfully request withdrawal of the rejection.

Rejection of Claims 1-9 Under 35 U.S.C. §102(a)

Claims 1-9 stand rejected under 35 U.S.C. §102(a) as allegedly anticipated by Waner *et al.* Applicants respectfully traverse.

Each of the independent claims are now directed to compositions of matter and articles of manufacture that include an isolated polypeptide *consisting essentially of* the polypeptide shown in SEQ ID NO:2, a phenotypically silent amino acid substitution variant of SEQ ID NO:2 that specifically binds to an anti-*Ehrlichia* antibody, and a conservative amino acid substitution variant of SEQ ID NO:2 that specifically binds to an anti-*Ehrlichia* antibody.

Waner does not teach or suggest the use of distinct *E. canis* polypeptides as shown in SEQ ID NO:2. That is, Waner does not teach or suggest an invention that includes a polypeptide consisting essentially of the about 20 amino acid polypeptide of SEQ ID NO:2 or the specified variants of SEQ ID NO:2 that specifically bind to an anti-*Ehrlichia* antibody. Instead, Waner teaches an IFA for *E. canis* that uses DH82 cells that are heavily infected with *E. canis* as an antigen or an ELISA with an *E. canis* antigen. See page 240, second column, last paragraph (IFA) and page 241, first column, first full paragraph. (ELISA). Waner, therefore, teaches entire cells or whole proteins as assay antigens, not polypeptides *consisting essentially of* the about 20 amino acid polypeptide of SEQ ID NO:2 or the specified variants of SEQ ID NO:2 that specifically bind to an anti-*Ehrlichia* antibody. The claims of the present invention and the subject matter contemplated by Waner do not read on each other.

Regardless whether one in the art possessed knowledge of the exact sequence of *E. canis* antigen, one of skill in the art would have known, at a minimum, that infection with *E. canis* whole

protein necessarily implicated a protein of at least 20kD in mass. See, for example, Brouqui P., Antigenic characterization of *Ehrlichiae*: protein immunoblotting of *Ehrlichia canis*, *Ehrlichia sennetsu*, and *Ehrlichia risticii*. J Clin Microbiol 1992 May; 30(5): 1062-6; attached. (The smallest antigenic peptide in *E. canis* associated with a tick borne illness is 20kD). Knowing the sequence of P30 would reveal an approximately 30kD protein. SEQ ID NO:1 and SEQ ID NO:2 are each just more than 2kD. Consequently, it is impossible for the whole proteins of Waner to comprise "polypeptides having at least 85% identity, more preferably at least 90% identity, and still more preferably at least 96%, 97%, 98%, or 99% identity to a polypeptide sequence shown in SEQ ID NOs:1-7" as recited in the specification. See, e.g., page 5, lines 7-13. Even a tandem or triplet repeat of, for example, SEQ ID NO:2, as contemplated in the specification (page 10, line 21 through page 11, line 6), cannot possibly share at least 85% identity, more preferably at least 90% identity, and still more preferably at least 96%, 97%, 98%, or 99% identity with whole length protein. In fact, an alignment between the *E. canis* P30 protein and SEQ ID NO:2 reveals 6.9% identity. See Appendix B, hereto. Thus, contrary to the Examiner's assertion, there <u>is</u> evidence that the claimed composition and article of manufacture differs from Waner.

Furthermore, the results presented, *inter alia*, page 19, line 17 through page 21, line 6, and Table 2, indicate that unlike the whole protein-based assays of Waner, the embodiments of the present invention enable unexpectedly sensitive and selective testing for *E. canis* antibody, more sensitive and selective than either the alleged "gold standard" of IFA (see Waner, abstract) or the ELISA assay of Waner. For example, the Waner ELISA assay was about 16% less sensitive than IFA at seven days post infection (4/6 positives versus 5/6 positives) but finished approximately equivalent to the IFA assay. See Waner, page 242, left column, first full paragraph. In contrast, utilizing the peptides contemplated in the present invention, five true negatives were correctly identified as such where the same samples were falsely identified as positive by IFA. See, e.g., Table 2. Further, seven true positives identified as such utilizing the peptides contemplated in the present invention were falsely identified as negative by IFA. See, e.g., Table 2. Fifty-seven true positives were properly identified by both assays. Thus, the embodiments of the present invention allow

unexpectedly sensitive and selective assays for *E. canis* antibody, which are not anticipated by the teachings of Waner.

Waner does not identify the polypeptide fragments to be of any particular use. There is no teaching in Waner, directly or inherently, that would direct one of skill in the art to an invention consisting essentially of the about 20 amino acid polypeptide of SEQ ID NO:2 or the specified variants of SEQ ID NO:2 that specifically bind to an anti-*Ehrlichia* antibody. Warner does not teach or suggest that polypeptides of SEQ ID NO:2 would be useful as individual polypeptides apart from entire *E. canis* infected cells or entire proteins. Warner provides no recognition or suggestion that the distinct polypeptides shown in SEQ ID NO:2 or the specified variants or any other polypeptide fragments would be of diagnostic use.

Furthermore, Waner combined with the other references cited in the Office Action does not render obvious the present invention because there is no teaching or suggestion that the use of SEQ ID No:2 or conservative amino acid substitution variants or phenotypically silent amino acid substitution variants thereof that specifically binds to an anti-*Ehrlichia* antibody would enable specificity and sensitivity significantly beyond that available even in the alleged "gold standard" of IFA. The polypeptides of the present invention possess unexpectedly and significantly enhanced and improved function, such that the use of a whole protein does not so anticipate.

Additionally, the Office Action is relying on an inherency theory to sustain this anticipation rejection. While Waner may inherently describe a full length protein, there is nothing in Waner that inherently discloses the present invention of an about 20 amino acid sequence which provides superior binding to an anti-*Ehrlichia* antibody than the full length sequence inherent in Waner.

Waner does not anticipate claims 1-9 because Waner does not teach, suggest, or inherently disclose each and every element of claims 1-9. Further, Waner does not teach, suggest or inherently disclose the unexpectedly enhanced function afforded by the sequences contemplated in the present invention. Thus, Applicants respectfully request withdrawal of the rejection.

Rejection of Claims 1-9 Under 35 U.S.C. §102(b)

Claims 1-9 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by Cadman *et al.* The Office Action asserts that the polypeptide compositions and article of manufacture of the invention are inherently present in the assays disclosed in Cadman. Applicants respectfully traverse.

Cadman does not teach or suggest the use of distinct *E. canis* polypeptides *consisting essentially of* the polypeptides shown in SEQ ID NO:2 and the claimed variants. Cadman teaches an IFA for *Ehrlichia canis* that uses DH82 cells which are heavily infected with *E. canis* as an antigen. See Cadman, first column, fourth paragraph. Cadman also teaches a dot-blot enzyme linked immunoassay (DBELIA) for *E. canis* that uses an *E. canis* antigen purified from infected DH82 cells. See Cadman, first column, fifth paragraph. As such, Cadman teaches the use of whole *E. canis* infected cells or whole proteins purified from *E. canis* infected cells in the disclosed assays. Cadman does not teach, suggest, or inherently disclose that any specific polypeptide fragments are of any particular use, nor does it teach, suggest, or disclose polypeptides *consisting essentially of* the about 20 amino acid polypeptide of SEQ ID NO:2 or the claimed variants of SEQ ID NO:2 that specifically bind to an anti-*Ehrlichia* antibody.

In addition, is impossible for the whole proteins of Cadman to comprise "polypeptides having at least 85% identity, more preferably at least 90% identity, and still more preferably at least 96%, 97%, 98%, or 99% identity to a polypeptide sequence shown in SEQ ID NOs:1-7" as recited in the specification. Even a tandem or triplet repeat of, for example, SEQ ID NO:2 cannot possibly share at least 85% identity, more preferably at least 90% identity, and still more preferably at least 96%, 97%, 98%, or 99% identity with whole length protein. See, e.g., page 5, lines 7-13. As shown in Appendix B, hereto, an alignment between the *E. canis* P30 protein and SEQ ID NO:2 reveals 6.9% identity. Thus, there is evidence that the claimed composition and article of manufacture differs from Cadman.

Furthermore, as presented above, the results, *inter alia*, page 19, line 17 through page 21, line 6, and Table 2, indicate that unlike the whole protein-based assays of Cadman, the embodiments of the present invention enable unexpectedly sensitive and selective testing for *E. canis* antibody, more sensitive and selective than either IFA or the DBELIA assay of Cadman. For example, the

Cadman DBELIA assay was only about 92% as sensitive and 96% as specific as IFA. As explained above, the polypeptides of the present invention provide results superior to the "gold standard" IFA. *See* Cadman, page 362, paragraph bridging the columns. For example, utilizing the peptides contemplated in the present invention, 5 true negatives were identified as such where the same samples were falsely identified as positive by IFA. Further, 7 true positives identified as such utilizing the peptides contemplated in the present invention were falsely identified as negative by IFA. Fifty-seven true positives were properly identified by both assays. Thus, the embodiments of the present invention allow unexpectedly sensitive and selective assays for *E. canis* antibody, which are not anticipated by the teachings of Cadman.

In addition, there is no teaching in Cadman, directly or inherently, that would direct one of skill in the art to sequences *consisting essentially of* the about 20 amino acid polypeptide of SEQ ID NO:2 or the specified variants of SEQ ID NO:2 that specifically bind to an anti-*Ehrlichia* antibody. Cadman does not teach or suggest that polypeptides of SEQ ID NO:2 or specified variants would be useful as individual polypeptides apart from entire *E. canis* infected cells or entire proteins. Cadman provides no recognition or suggestion that the distinct polypeptides shown in SEQ ID NO:2, specified variants, or any other polypeptide fragments would be of use.

In addition, while Cadman may inherently describe a full-length protein, there is nothing in Cadman that inherently discloses the present invention of an about 20 amino acid sequence which provides superior binding to an anti-*Ehrlichia* antibody than the full-length sequence inherent in Waner.

Cadman does not teach each and every element of the claimed invention. Further, Cadman does not teach, suggest or inherently disclose the unexpectedly enhanced function afforded by the sequences contemplated in the present invention. Therefore, Cadman does not anticipate the claimed invention. Applicants respectfully request withdrawal of the rejection.

Rejection of Claims 1-9 Under 35 U.S.C. §102(b)

Claims 1-9 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by Rikihisa et al. (WO 99/13720). Applicants respectfully traverse.

The Final Office Action asserts that Rikihisa teaches the polypeptide of SEQ ID NO:2, and that an article of manufacture is inherent in the teachings of Rikihisa. However, Rikihisa does not teach or suggest the use of polypeptides *consisting essentially of* the about 20 amino acid polypeptide of SEQ ID NO:2 or phenotypically silent or conservative amino acid substitution variants of SEQ ID NO:2 that specifically bind to an anti-*Ehrlichia* antibody. Rather, Rikihisa relates to a complete 288 amino acid protein sequence. In addition, is impossible for the whole proteins of Rikihisa to comprise "polypeptides having at least 85% identity, more preferably at least 90% identity, and still more preferably at least 96%, 97%, 98%, or 99% identity to a polypeptide sequence shown in SEQ ID NOs:1-7" as recited in the instant specification. As shown in Appendix B, hereto, an alignment between the *E. canis* P30 protein and SEQ ID NO:2 reveals 6.9% identity. Thus, there is evidence that the claimed composition and article of manufacture differs from Rikihisa. Therefore, Rikihisa does not teach, suggest, or inherently disclose the specific, individual polypeptides shown in SEQ ID NO:2 or its specified variants and does not identify the polypeptide fragments to be of any particular diagnostic use. The claims of the present invention and the subject matter contemplated in Rikihisa do not read on each other.

Further, there is no teaching in Rikihisa, directly or inherently, that would *direct* one of skill in the art to the particular polypeptides consisting essentially of the about 20 amino acid polypeptide of SEQ ID NO:2 or phenotypically silent or conservative amino acid substitution variants of SEQ ID NO:2 that specifically bind to an anti-*Ehrlichia* antibody. Rikihisa does not teach or suggest that polypeptides of the instant invention would be useful as individual polypeptides apart from the entire *E. canis* P30 protein. Rikihisa provides no recognition or suggestion that the distinct polypeptides shown in SEQ ID NO:2 or its specified variants or any other polypeptide fragments would be of any use. More importantly, Rikihisa provides no teaching or suggestion, let alone anticipates, that polypeptides and variants of the invention would enable unexpectedly sensitive and selective testing for *E. canis* antibody, significantly more sensitive and selective than even IFA. Thus, the embodiments of the present invention allow unexpectedly sensitive and selective assays for *E. canis*

for *E. canis* antibody, significantly more sensitive and selective than even IFA. Thus, the embodiments of the present invention allow unexpectedly sensitive and selective assays for *E. canis* antibody, which are neither anticipated by the teachings of Rikihisa nor suggested upon combination with any other references cited against the instant invention.

Rikihisa does not teach each and every element of the claimed invention and it does not teach, suggest or inherently disclose the unexpectedly enhanced function afforded by the sequences contemplated in the present invention. Therefore, Rikihisa does not anticipate the claimed invention. Applicants respectfully request withdrawal of the rejection.

Conclusion

Applicants respectfully submit that the claims are in a condition for allowance. If the Examiner of the opinion that that a telephone conference would expedite the prosecution of the application, the Examiner is encouraged to contact Applicants undersigned representative.

Respectfully submitted,

Date: 3/24/03 by: _____

Jeffrey Anderson
Reg. No. 51,403

Appendix A
Marked-Up Version of the Amendments to Show Changes Made

1. (Twice Amended) A composition of matter consisting essentially of an isolated polypeptide shown in SEQ ID NO:2 or a phenotypically silent amino acid substitution variant thereof **that specifically binds to an anti-*Ehrlichia* antibody**.

2. (Amended) The composition of claim **[2] 1**, further comprising a carrier.

3. (Twice Amended) An article of manufacture comprising packaging material and, contained within the packaging material, a polypeptide **consisting essentially of the polypeptide** shown in SEQ ID NO:2 or a phenotypically silent amino acid substitution variant thereof **that specifically binds to an anti-*Ehrlichia* antibody**.

5. (Twice Amended) The article of manufacture of claim 4, wherein the label indicates that identification of an *Ehrlichia* infection is done using a method of detecting presence of antibodies to *Ehrlichia* comprising:

(a) contacting a polypeptide **consisting essentially of the polypeptide** shown in SEQ ID NO:2 or a phenotypically silent amino acid substitution variant of the polypeptide shown in SEQ ID NO:2 **that specifically binds to an anti-*Ehrlichia* antibody**, with a test sample suspected of comprising antibodies to *Ehrlichia*, under conditions that allow polypeptide/antibody complexes to form; and

(b) detecting polypeptide/antibody complexes;

wherein the detection of polypeptide/antibody complexes is an indication that an *Ehrlichia* infection is present.

7. (Amended) A composition of matter consisting essentially of an isolated polypeptide shown in SEQ ID NO:2 or a conservative amino acid substitution variant thereof **that specifically binds to an anti-Ehrlichia antibody**.

8. (Amended) An article of manufacture comprising packaging material and, contained within the packaging material, a polypeptide **consisting essentially of the polypeptide** shown in SEQ ID NO:2 or a conservative amino acid substitution variant thereof **that specifically binds to an anti-*Ehrlichia* antibody**.

9. (Amended) The article of manufacture of claim 4, wherein the label indicates that identification of an *Ehrlichia* infection is done using a method of detecting presence of antibodies to *Ehrlichia* comprising:

(a) contacting a polypeptide **consisting essentially of the polypeptide** shown in SEQ ID NO:2 or a conservative amino acid substitution variant of the polypeptide shown in SEQ ID NO:2 **that specifically binds to an anti-*Ehrlichia* antibody**, with a test sample suspected of comprising antibodies to *Ehrlichia*, under conditions that allow polypeptide/antibody complexes to form; and

(b) detecting polypeptide/antibody complexes;

wherein the detection of polypeptide/antibody complexes is an indication that an *Ehrlichia* infection is present.

Appendix B
Alignment between SEQ ID NO:2 and the *E. canis* P30 protein

From http://xylian.igh.cnrs.fr/

GeneStream align Home Page
align Search Help

align Results
Please site: *Pearson, W.R., Wood, T., Zhang, Z., and Miller, W. (1997) Comparison of DNA sequences with protein sequences, Genomics 46: 24-36*

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Ehrlichia canis P30                                  288 aa vs.
SEQ ID NO:2                                           20 aa
scoring matrix: , gap penalties: -12/-2
6.9% identity;          Global alignment score: -419

        10        20        30        40        50        60
MNCKRFFIASALISLMSFLPSVSFSESIHEDNINGNFYISAKYMPSASHFGVFSVKEEKN
                                                           :
-----------------------------------------------------------N


        70        80        90       100       110       120
TTTGVFGLKQDWDGATIKDASSSHTIDPSTIFSISNYSFKYENNPFLGFAGAIGYSMGGP
::::::::::::::::::
TTTGVFGLKQDWDGATIKD-----------------------------------------
        10         20

       130       140       150       160       170       180
RVEFEVSYEIFDVKNQGNSYKNDAHKYCALSRHTGGMPQAGHQNKFVFLKNEGLLDISLM

------------------------------------------------------------


       190       200       210       220       230       240
INACYDITIDSMPFSPYICAGIGSDLVSMFETTNPKISYQGKLGVSYSISPEASVFVGGH

------------------------------------------------------------


        250       260       270       280
FHRVIGNEFKDIPAITPAGATEIKGTQFTTVTLNICHFGLELGGRFTF

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